Mononucleosomes H2A Ubiquityl, Recombinant Human, Biotinylated

Catalog No. 16-0020

Lot No. 17209001

Pack Size 50 μg

Product Description:

Produced in collaboration with Boston Biochem®, this mononucleosome is assembled from recombinant human histones expressed in E. coli (two each of histones H2A, H2B, H3 and H4. Accession numbers: H2A-P04908; H2B-O60814; H3.1-P68431; H4-P62805) wrapped by 147 base pairs of 601 positioning sequence DNA. There is a 5' biotin-TEG group on the DNA. Approximately 50% of the nucleosomes are mono-ubiquitinated on histone H2A lysine 118, while the other 50% are mono-ubiquitinated on both histone H2A lysine 118 and histone H2A lysine 119 (multi-mono-ubiquitinated). Polycomb Repressive Complex 1 (PRC1) is a multi-subunit protein complex that is essential for maintaining epigenetic repression of key developmental loci in embryonic stem cells (ESCs). PRC1 proteins BMI1, RNF2 and others function as a Ubiquitin E3 ligase that specifically mono-ubiquitinates histone H2A (H2AK119ub1) which effects chromatin remodeling in a heritably transmissible manner. BMI1 activity has also been reported as necessary for constitutive heterochromatin formation in mammalian somatic cells.

Formulation:

Mononucleosomes H2A Ubiquityl, Recombinant Human, Biotinylated (27.3 µg protein weight, 50 µg total weight) in 43.9 µl of 25 mM Tris pH 7.5, 150 mM NaCl, 2 mM DTT, 20% glycerol. Molarity = 5.02 µmolar. MW = 227,000 Da.

Storage and Stability:

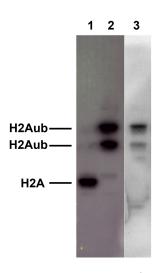
Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid multiple freeze/thaws.

Application Notes:

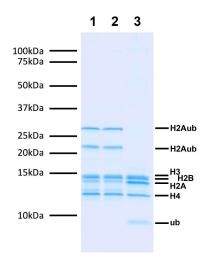
Mononucleosomes H2A Ubiquityl, Recombinant Human, Biotinylated are useful as substrates in deubiquitylation (Dub) assays and for inhibitor screening.

References Using this Product:



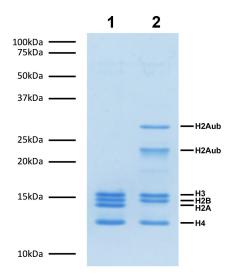


Western Blot Data: Western blot analysis of Mononucleosomes H2A ubiquityl, Recombinant Human, Biotinylated, probed with either an antibody to histone H2A (left panel, lanes 1 and 2) or an anti-ubiquitin antibody (right panel, lane 3). Lane 1 contains the unmodified recombinant nucleosome, Lanes 2 and 3 contain the H2A-ubiqutylated nucleosome.

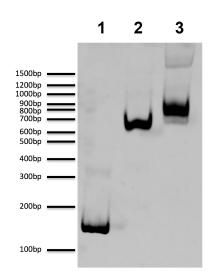


Deubiquitylation Assay Data: Mononucleosomes H2A Ubiquityl, Recombinant Human, Biotinylated (1 μg) were employed in a deubiquitylation (Dub) assay using no enzyme (Lane 1), USP5 (Lane 2) or USP16 (Lane 3) and run on an SDS PAGE gel. Only the USP16-treated sample shows the elimination of H2Aub bands, the appearance of monoubiqutin (ub) and the reappearance of H2A.

This product is for in vitro research use only and is not intended for use in humans or animals.



Protein Gel Data: Coomassie stained PAGE gel of proteins in Mononucleosomes H2A ubiquityl, Recombinant Human, Biotinylated (1 μg) to demonstrate the purity of the histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2Aub, H2B, H3 and H4) are indicated. Lane 1: Unmodified recombinant nucleosomes. Lane 2: Mononucleosomes H2A ubiquityl, Recombinant Human, Biotinylated.



DNA Gel Data: Mononucleosomes H2A ubiquityl, Recombinant Human, Biotinylated run on a native PAGE gel and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA extracted from nucleosomes (200 ng). **Lane 2:** Intact unmodified recombinant nucleosomes (400 ng). **Lane 3:** Mononucleosomes H2A ubiquityl, Recombinant Human, Biotinylated (400 ng).